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Asymmetric preparation of antifungal 1-(4′-chlorophenyl)-1-cyclopropyl methanol and 1-(4′-chlorophenyl)-2-phenylethanol. Study of the detoxification mechanism by *Botrytis cinerea*

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ABSTRACT

Chiral alcohols are important as bioactive compounds or as precursors to such molecules. On the basis of the different antifungal properties of the enantiopure alcohol derivatives of 4′-chlorophenyl cyclopropyl ketone and benzyl 4′-chlorophenyl ketone, their enantioselective synthesis by chemical and biocatalytic methods was studied. The detoxification pathways by the phytopathogen fungus *Botrytis cinerea* are reported.

1. Introduction

Botrytis cinerea has been identified as a pathogenic fungus associated with over 235 plant species including grapes, lettuce, tomatoes, tobacco, and strawberries. It grows as a grey mould causing serious economic losses [1,2]. Since several strains have developed resistance to some commercial fungicides [3], there is an important need to develop novel antifungal agents that are active against this organism.

On the basis of previous results indicating that the presence of a hydroxyl group was fundamental for the expression of antifungal activity against the phytopathogenic fungus *B. cinerea* [4–7], we showed that the alcohol derivatives of 4′-chlorophenyl cyclopropyl ketone (**1**) and benzyl 4′-chlorophenyl ketone (**2**), compounds analogous to several phytoalexins [8,9], are active fungistatic agents against *B. cinerea* and *Colletotrichum gloeosporioides* [10].

The development of highly effective systems for the synthesis of chiral alcohols is not only of interest to the academic world but also has attracted the attention of industrial scientists. Because of their environmentally benign reaction conditions and unparalleled chemo, regio, and stereoselectivities, enzymatic protocols have been gaining in popularity [11]. In this paper we report on the enantioselective synthesis of 1-(4′-chlorophenyl)-1-cyclopropyl methanol (**3**) and 1-(4′-chlorophenyl)-2-phenylethanol (**4**) in both absolute configurations by chemical and biocatalytic methods.

Since several phytopathogenic fungi have been reported to detoxify phytoalexins [12] producing less toxic degradation products than the parent compounds [13], we decided to study the biotransformation of 1-(4′-chlorophenyl)-1-cyclopropyl methanol (**3**) by *B. cinerea* as a part of the fungal detoxification mechanism. Previous studies of the detoxification of 1-(4′-chlorophenyl)-2-phenylethanol (**4**) by *B. cinerea* and *C. gloeosporioides* suggest that it is not likely to persist in the environment for long periods post-application [14].

2. Experimental

2.1. General experimental procedures

Optical rotations were determined with a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Mattson Genesis spectrophotometer, series FTIR. ^{1}H and ^{13}C NMR measurements were obtained on Varian Unity 400 and Varian Unity 600 NMR spectrometers with $SiMe_4$ as the internal reference. Mass spectra were recorded on a GC–MS Thermoquest spectrometer (model: Voyager), and a VG Autospec-Q spectrometer. HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with a UV-VIS

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detector (L 6200) and a differential refractometer detector (RI-71). TLC was performed on Merck Kiesegel 60 F_{254}, 0.2 mm thick. Silica gel (Merck) was used for column chromatography. Purification by means of HPLC was accomplished with a silica gel column (Hibar 60, 7 m, 1 cm wide, 25 cm long). Chemicals were produced by Fluka or Aldrich. All solvents used were freshly distilled. Baker's yeast was obtained from a local store. The following enzymes were used in this work: *Candida rugosa* lipase (CRL, Sigma, Type VII, 950 U/mg), *Pseudomonas cepacia* lipase (PSL, Amano Pharmaceuticals Co., Japan) and porcine pancreas lipase (PPL, Sigma, Type II).

2.2. Chemical transformations

2.2.1. Synthesis of racemic substrates

Compounds 4′-chlorophenyl cyclopropyl ketone (**1**) (2 g, 0.012 mol) and benzyl 4-chlorophenyl ketone (**2**) (2 g, 0.008 mol) were treated with $NaBH_4$ (0.8 g, 0.02 mol) in methylene chloride:methanol 1:1 (400 mL) at room temperature stirring for 24 h. Distillation under reduced pressure to eliminate the solvent led to a crude mixture that was neutralised with aqueous HCl 10% and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and the solvent was eliminated by means of distillation under reduced pressure. The reduction mixture was chromatographed on a silica gel column eluting with hexane–ethyl acetate mixtures to give (±)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (**3**) (1.98 g, 98%) and (±)-1-(4′-chlorophenyl)-2-phenylethanol (**4**) (1.85 g, 91%), respectively. The ^{1}H NMR spectra of these products were in agreement with those found in the literature [15,16].

After purification, compounds **3** (1.44 g, 0.006 mol) or **4** (1.5 g, 0.008 mol) were dissolved in dry pyridine and acetic anhydride (50 mL) was added dropwise. The reaction mixtures were stirred for 20 h. The solvent was then removed and the crude reaction product chromatographed to give (±)-1-(4′-chlorophenyl)-1-cyclopropyl methyl acetate (**5**) (94.1%) and (±)-1-(4-chlorophenyl)-2-phenylethyl acetate (**6**) [14] (93.2%).

(±)-1-(4′-chlorophenyl)-1-cyclopropyl methyl acetate (5). Obtained as a colourless oil. IR ν_{max} (film): 2925, 1738, 1615, 1371, 1090, 818; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.36 (m, 2H, H-3), 0.54 (m, 2H, H-4), 1.25 (m, 1H, H-2), 2.09 (s, 3H, –COOC*H*$_3$), 5.17 (d, 2H, J_{1-2} = 8.7 Hz, H-1), 7.31 (bs, 4H, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 3.0 (t, C-4), 4.1 (t, C-3), 16.4 (d, C-2), 21.2 (c, –COO*C*H$_3$), 78.4 (d, C-1), 127.9 (d, C-2′, C-6′), 128.5 (d, C-3′, C-5′), 133.5 (s, C-4′), 138.8 (s, C-1′), 170.2 (s, C=O). MS (*m*/*z*, %): 226 (M^++2, 2), 224 (M^+, 5), 164 (M^+−60, 71), 138 (100), 77 (50).

Enantiomeric excesses were determined by means of HPLC analyses on a chiral column (Chiralcel OD, Daicel, Japan): 254 nm, 0.5 mL/min, hexane:isopropanol (95:5) (*R*)-**3** t_R = 18.6 min, (*S*)-**3** t_R = 21.6 min; 0.8 mL/min, hexane:isopropanol (97:3) (*S*)-**4** t_R = 20.3 min, (*R*)-**4** t_R = 29 min; 0.7 mL/min, hexane:isopropanol (99.5:0.5) (*R*)-**5** t_R = 9 min, (*S*)-**5** t_R = 14 min.; 0.7 mL/min, hexane:isopropanol (99.5:0.5) (*S*)-**6** t_R = 8 min, (*R*)-**6** t_R = 10 min.

2.2.2. Synthesis of enantiomeric alcohols

2.2.2.1. (S)-(−)-1-(4′-chlorophenyl)-1-cyclopropylmethanol (S)-3. Under an argon atmosphere, an oven-dried Schlenk tube was charged with (*S*)-methyl oxazaborolidine (0.086 mmol, 86 μL of 1 M solution in toluene) [17]. The solvent was removed under high vacuum conditions (0.1 mbar) at room temperature and tetrahydrofuran (THF, 11 mL) was added. Once the solution was cooled to 0 °C, it was treated with a borane–THF complex (1.0 M, 1.29 mL) and stirred at room temperature for 2 h. This reagent and a solution of 4′-chlorophenyl cyclopropyl ketone (**1**) (155 mg, 0.997 mmol) in THF (10 mL) were then added simultaneously from two syringes into an oven dried, round-bottomed flask at a temperature of 30 °C over a period of 14 h. After the reaction mixture was stirred for an additional 1 h, water (20 mL) was added, the organic solvent was removed by means of rotary evaporation and the remaining aqueous phase was extracted with diethyl ether. The combined organic layers were then dried over magnesium sulphate. Removal of the solvent by means of rotary evaporation and subsequent filtration through a short silica gel column afforded 153.9 mg (98.1%) of the (*S*)-alcohol ($[\alpha]_D^{20} = -9.1°$ (*c* 2.9 in $CHCl_3$), 40.1% *ee*).

2.2.2.2. (R)-(+)-1-(4′-chlorophenyl)-1-cyclopropylmethanol (R)-3 and (R)-(+)-1-(4′-chlorophenyl)-2-phenylethanol (R)-4. Under an argon atmosphere, an oven-dried Schlenk tube was charged with (*R*)-methyl oxazaborolidine (0.086 mmol, 100 μL of 1 M solution in toluene). The solvent was removed under high vacuum conditions (0.1 mbar) at room temperature and tetrahydrofuran (THF, 11 mL) was added. The solution was then cooled to 0 °C, treated with a borane–THF complex (1.0 M, 1.49 mL) and stirred at room temperature for 2 h. This reagent and a solution of 4′-chlorophenyl cyclopropyl ketone (**1**) (180 mg, 0.996 mmol) or benzyl 4′-chlorophenyl ketone (**2**) (230 mg, 1.0 mmol), respectively, in THF (10 mL), were then added simultaneously from two syringes into an oven dried, round-bottomed flask at a temperature of 30 °C over a period of 16 h. The work-up procedure was essentially the same as that described above affording 175.3 mg (96.3%) of (*R*)-**3** ($[\alpha]_D^{20} = +9.4°$ (*c* 2.1 in $CHCl_3$), 45.2% *ee*) and 227 mg (98%) of (*R*)-**4** ($[\alpha]_D^{20} = +23.3°$ (*c* 1.2 in $CHCl_3$), >99% *ee*), respectively.

2.2.3. Treatment of 1-(4′-chlorophenyl)-1-cyclopropylmethanol (3) in Czapek-Dox medium

The compound (±)-1-(4′-chlorophenyl)-1-cyclopropylmethanol (**3**) (10 mg, 0.055 mmol) in 100 mL of Czapek-Dox medium at pH 4 was stirred at room temperature for 120 h without the fungus *B. cinerea*. No reaction products were obtained.

2.3. General procedure for the baker's yeast transformation

A mixture composed of baker's yeast (250 g), D-glucose (100 g), and tap water (1 L) was stirred in a 2 L beaker at 50 °C for 30 min. The substrate (2 g), dissolved in the minimum amount of ethanol, was then added dropwise. At the end of the reaction period, 1 L of ethyl acetate was added and the crude reaction mixture filtered through a large Büchner funnel on a Celite pad, which was later washed with the same solvent. The aqueous phase was extracted twice with 0.5 L of ethyl acetate, the organic phase dried over Na_2SO_4, and the solvent then evaporated under reduced pressure to dryness. The residue obtained was purified by means of column chromatography.

2.3.1. Baker's yeast transformation in the presence of several additives

A mixture of baker's yeast (250 g), water (1 L), and D-glucose (100 g, 0.505 mol) was stirred at 50 °C for 30 min. The substrate (2 g) was dissolved in 8 mL of allyl alcohol (67 mmol) and 12 mL of hexane (1.2%) and was then added dropwise to the mixture [18]. The reaction was then stirred for a further period of time. The work-up procedure was essentially the same as that described above. The results are shown in Table 1.

2.4. Lipase-mediated reactions

2.4.1. Lipase-mediated acetylations

A mixture of racemic alcohol **3** or **4** (50 mg), lipase (50 mg), and vinyl acetate in *tert*-butylmethyl ether (TBME) (2 mL) was stirred at room temperature. The residue obtained upon evaporation of the filtered reaction mixture was chromatographed on a silica gel col-

Table 1
Results of baker's yeast-mediated reduction.

Substrate	Allyl alcohol (mM)	Hexane (%)	Conversion[a] (%)	*ee* (%)	Time (h)
1	–	–	5.0	27	72
	67	1.2	12.0	96	96
2	–	–	1.0	50	72
	–	–	15.3	67	96
	67	1.2	2.0	99	168
	67	1.2	3.7	94	240

[a] % of product in the recovered material.

Table 2
Results of lipase-mediated acetylation.

Substrate	Enzyme	Vinyl acetate (mmol)	Temp. (°C)	Time (h)	Yield$_p$ (%)[a]	*ee*$_p$ (%)	Yield$_s$ (%)	*ee*$_s$ (%)	Conversion (%)	*E*
3	PSL	2.3	RT	72	18.0	97	78.4	88	47.6	190
	PSL	0.23	RT	47.5	1.0	74	85.6	78	51.3	16
	PSL	10	RT	240	29.0	90	84.0	18	16.5	24
	PPL	10	RT	240	10.0	>99	93.0	7	6.6	>200
4	PSL	0.23	RT	96	2.7	54	87.3	24	31.0	4
	PSL	0.23	RT	168	15.3	67	80.0	42	38.5	8
	PSL	10	48	264	10.0	70	94.0	6	6.0	6
	PPL	10	40	264	10.0	97	94.0	6	6.0	73

[a] Yield 100% at 50% conversion after hydrolysis of the acetate derivative.

umn and eluted with hexane:ethyl acetate (95:5). The first eluted fractions provided the acetate derivatives (*R*)-**5** ($[\alpha]_D^{20} = +1$ (*c* 1.1 in $CHCl_3$), >99% *ee*) and (*R*)-**6** ($[\alpha]_D^{20} = +5$ (*c* 1.7 in $CHCl_3$), 97% *ee*) while the last afforded the unreacted starting material. Detailed results of the enzyme-mediated acetylations are reported in Table 2.

Treatment of acetate derivatives (*R*)-**5** (300 mg, 1.32 mmol) and (*R*)-**6** (375 mg, 1.4 mmol), respectively, with KOH (100 mg, 2 mmol) in methanol solution at room temperature for 2 h afforded 239.4 mg (97.8%) of the compound (*R*)-(+)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (*R*)-**3** ($[\alpha]_D^{20} = +21^\circ$ (*c* 2.7 in $CHCl_3$), >99% *ee*) and 321.2 mg (98.8%) of (*R*)-(+)-1-(4′-chlorophenyl)-2-phenylethanol (*R*)-**4** ($[\alpha]_D^{20} = +22^\circ$ (*c* 1.6 in $CHCl_3$), 97% *ee*), respectively.

2.4.2. Lipase-mediated hydrolysis

Lipase (90 mg of PSL, 60 mg of PPL or CRL) was added to a mixture of racemic acetates **5** or **6** (0.075 mmol) in 1.5 mL of solvent (mixture of 1,4-dioxane and buffer KH_2PO_4:KOH 0.1 M pH 7). The solution was stirred at room temperature. The residue obtained upon evaporation of the filtered reaction mixture was chromatographed on a silica gel column and eluted with hexane:ethyl acetate (95:5). The first eluted fractions provided the unreacted starting material and the last eluted fractions afforded the alcohol derivatives (*R*)-(+)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (*R*)-**3** ($[\alpha]_D^{20} = +20^\circ$ (*c* 1.3 in $CHCl_3$), 98% *ee*) and (*R*)-(+)-1-(4′-chlorophenyl)-2-phenylethanol (*R*)-**4** ($[\alpha]_D^{20} = +4.8^\circ$ (*c* 1.0 in $CHCl_3$), 21.3% *ee*), respectively. Detailed results are reported in Table 3.

2.5. Microorganism culture and antifungal assays

The culture of *B. cinerea* employed in this work, *B. cinerea* UCA992, was obtained from grapes from the Domecq vineyard, Jerez de la Frontera, Cádiz, Spain. This culture is deposited in the Mycological Herbarium Collection (Universidad de Cádiz).

Antifungal activity was determined by means of a conidial germination assay. The test compound was dissolved in the minimum amount of ethanol, and water was then added to give a final compound concentration of 10–150 mg L^{-1}. Two microlitres of a conidial suspension in water containing about 5×10^4 conidia/mL of the *B. cinerea* strain were added to a solution of the test compound (15 μL) and PDB (potato dextrose broth) medium (2 μL) on ELISA plates. The final ethanol concentration was identical in control and treated cultures. Three replicates were made per compound and the viability of conidia was estimated by measuring germination just after incubation at 25 °C for 24 h.

2.6. Biotransformation of (±)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (*3*) by B. cinerea UCA992

B. cinerea UCA992 was grown as a surface culture in Roux bottles at 25° C on a Czapek-Dox medium (150 mL per bottle) for 2 days. Substrate **3** was dissolved in ethanol and then distributed over 17 Roux bottles (100 ppm per bottle). Fermentation continued for a further period of 5 days. The mycelium was filtered and washed with brine and ethyl acetate. The broth was extracted three times with ethyl acetate and the extract dried over anhydrous sodium sulphate. The solvent was then evaporated and the residue was chromatographed first on a silica gel

Table 3
Results of lipase-mediated hydrolysis.

Substrate	Enzyme	Buffer (%)	Time (h)	Yield$_p$ (%)	*ee*$_p$ (%)	Yield$_s$ (%)	*ee*$_s$ (%)	Conv. (%)	*E*
5	PPL	83	192	38.0	96	65.0	88	47.7	142
	PSL	83	192	66.0	73	28.0	70	49.0	13
	CRL	67	480	76.0	71	21.0	40	36.0	9
	CRL	67	240	85.5	98	13.0	87	47.0	>200
	CRL	20	288	42.0	38	40.0	10	20.6	2
	PPL	20	288	17.0	36	71.0	23	39.0	3
	PSL	20	288	46.7	47	40.0	33	41.0	4
6	CRL	20	360	10.0	21.3	75.6	12	36.8	2

Conv., conversion.

column and then with HPLC with an increasing gradient of ethyl acetate to petroleum ether. The following compounds were isolated: recovered (±)-1-(4′-chlorophenyl)-1-cyclopropylmethanol (**3**) (207.5 mg), 4′-chlorophenyl cyclopropyl ketone (**1**) (2 mg) and di(4′-chlorophenyl cyclopropyl methyl)ether (**7**) (4 mg).

Di(4′-chlorophenyl cyclopropyl methyl)ether (**7**). Obtained as a colourless oil, *anti*/*syn* 50:50 as determined by ^{1}H NMR spectroscopy. IR ν_{max} (film): 2923, 1490, 1062, 817. MS (*m*/*z*, %): 346 (M^+, >0.1), 183 (2), 181 (5), 167 (32), 165 (100), 132 (2), 130 (35). *m*/*z* HRMS (EI, 70 eV) calcd. for $C_{20}H_{20}OCl_2$: 346.08912 $[M]^+$; found: 346.0913. *syn*-isomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.16 (m, 2H, H-3), 0.42 (m, 4H, H-3, H-4), 0.66 (m, 2H, H-4), 1.09 (m, 2H, H-2), 3.86 (d, 2H, J = 7.9 Hz, H-1), 7.19 (d, 4H, J = 8.3 Hz, H-2′, H-6′), 7.25 (d, 4H, J = 8.3 Hz, H-3′, H-5′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 1.6 (t, C-3), 5.0 (t, C-4), 17.3 (d, C-2), 81.7 (d, C-1), 128.2 (d, C-2′, C-6′), 128.4 (d, C-3′, C-5′), 133.0 (s, C-1′), 141.1 (s, C-4′). *anti*-isomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.08 (m, 2H, H-3), 0.21 (m, 2H, H-4), 0.36 (m, 2H, H-3), 0.60 (m, 2H, H-4), 1.13 (m, 2H, H-2), 3.25 (d, 2H, J = 8.2 Hz, H-1), 7.17 (d, 4H, J = 8.3 Hz, H-2′, H-6′), 7.31 (d, 4H, J = 8.3 Hz, H-3′, H-5′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 2.5 (t, C-3), 4.6 (t, C-4), 18.3 (d, C-2), 82.2 (d, C-1), 128.3 (d, C-2′, C-6′), 128.6 (d, C-3′, C-5′), 133.3 (s, C-1′), 140.4 (s, C-4′). HPLC (Chiralcel OD, Daicel, Japan, hexane/IPA 9.8:0.2, 0.6 mL/min): *anti*-t_R 7.3 min (*major*) and 9.7 min (*minor*); *syn*-t_R 6.7 min (*minor*) and 7.2 min (*major*).

3. Results and discussion

3.1. Chemical transformations

The chemical preparation of the (*S*)-enantiomer of 1-(4′-chlorophenyl)-2-phenylethanol (*S*)-**4** was previously reported with excellent yield and enantiomeric excess [14]. Thus, in order to obtain (*R*)-1-(4′-chlorophenyl)-2-phenylethanol (*R*)-**4** and both enantiomers of 1-(4′-chlorophenyl)-1-cyclopropyl methanol (**3**) [15], we employed the same chemical experimental procedure consisting of treatment of 4′-chlorophenyl cyclopropyl ketone (**1**) or benzyl 4′-chlorophenyl ketone (**2**) with BH_3–THF and (*R*)- or (*S*)-methyl oxazaborolidine [17]. Alcohol (*R*)-**4** was obtained with >99% *ee* and 98% yield but results in obtaining (*R*)- or (*S*)-**3** were moderate (45.2% *ee* for (*R*)-**3** and 40.1% *ee* for (*S*)-**3**). Enantiomeric excesses were determined by HPLC analysis using a Chiralcel OD column.

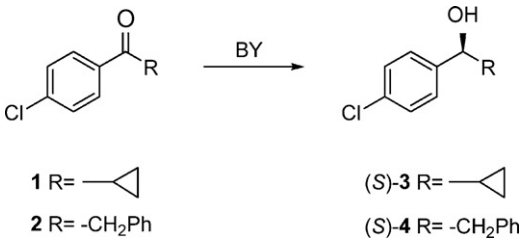
3.2. Biocatalytic transformations

3.2.1. Baker's yeast reduction

Based on the well known ability of baker's yeast fermentation to reductively transform a variety of ketones into optically active alcohols with (*S*)-configuration (Prelog specificity family) [18], we decided to use a baker's yeast-mediated approach to enantiopurify (*S*)-(−)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (*S*)-**3** and (*S*)-(−)-1-(4′-chlorophenyl)-2-phenylethanol (*S*)-**4**, using the corresponding ketones **1** and **2** as substrates. The results are shown in Table 1.

A major limitation of this methodology is the difficulty in extracting the product from the fermentation broth; consequently, often only moderate yields of products are obtained, presumably due to absorption of the starting material and/or product within the larger quantity of yeast cells [19,20].

As shown in Table 1, after the incubation of ketones **1** and **2** in fermenting baker's yeast following the conventional procedure (see Section 2), a single transformation product was isolated, (*S*)-(−)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (*S*)-**3** (5% conversion, $[\alpha]_D^{20} = -5.7^\circ$ (*c* 1.9 in $CHCl_3$), 27% *ee*) and (*S*)-1-(4′-chlorophenyl)-2-phenylethanol (*S*)-**4** (15.3% conversion, $[\alpha]_D^{20} = -15.4^\circ$ (*c* 1.5 in $CHCl_3$), 67% *ee*), respectively (Scheme 1). In order to improve



Scheme 1.

the transformation and the *ee*, we therefore tried to inhibit the reducing enzymes selectively by adding inhibitors such as allyl alcohol and a small amount of organic solvent [21] to dissolve the substrate (see Table 1). The addition of allyl alcohol and hexane improved the *ee* of both alcohols significantly, but the conversion itself was diminished for (*S*)-**4**: (*S*)-(−)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (*S*)-**3** (12% conversion, $[\alpha]_D^{20} = -20.1^\circ$ (*c* 0.9 in $CHCl_3$), 96% *ee*) and (*S*)-1-(4′-chlorophenyl)-2-phenylethanol (*S*)-**4** (2% conversion, $[\alpha]_D^{20} = -22^\circ$ (*c* 1.8 in $CHCl_3$), 99% *ee*), respectively. The spectroscopic data of these products corresponded to those found in the literature [15,16].

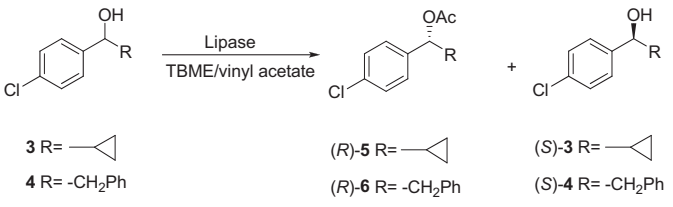
3.2.2. Lipase-mediated transformations

In order to study new methods for the preparation of the enantiomerically pure alcohols **3** and **4**, we focused on the kinetic resolution of the racemic secondary alcohols and acetates, obtained as described in the experimental section. Lipases are versatile biocatalysts acting with high efficiency in acylation or hydrolysis [11,22].

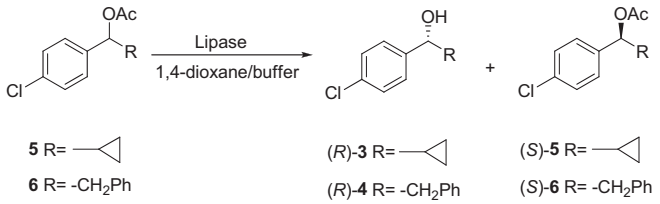
3.2.2.1. Lipase-mediated acetylations. The starting material **3** or **4** was added to different media with vinyl acetate as an acyl donor and *tert*-butyl methyl ether as the organic solvent and three different lipases: lipase PS (from *Pseudomonas cepacia*), PPL (porcine pancreas lipase) and CRL (*Candida rugosa* lipase). The lipases investigated converted the *R* enantiomers of **3** and **4** in accordance with Kazlauskas' empirical rule for secondary alcohols (Scheme 2) [23]. The results are summarised in Table 2.

The lipase PPL appeared to be more efficient for the enzymatic acetylation of these substrates affording the highest enantioselectivities. However, this enhanced selectivity was accompanied by a moderate reaction rate. CRL was rejected as a possible mediator due to the low selectivity and slow conversions observed when it was used. Lipase PSL showed high enantioselectivity and yield for compound **3** but low selectivity for alcohol **4**.

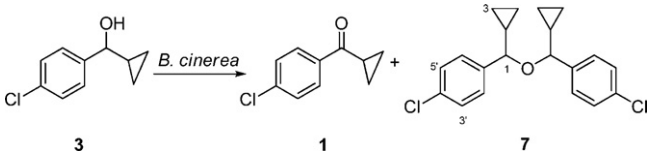
In an attempt to increase the low conversion rate and enantioselectivity showed for substrate **4** with all the lipases at room temperature, we also studied the effect of temperature on reaction conditions. We found that transesterification at the optimal temperature of each enzyme increased both parameters, conversion and enantiomeric excesses. This indicates that temperature may accelerate the kinetics of the enzyme. Different reaction times and vinyl acetate concentrations were also tested (see Table 2).



Scheme 2.



Scheme 3.



Scheme 4.

The best results for **3** and **4** were obtained with lipase PPL giving acetate derivatives with yields of 10% and >99% and 97% *ee*, respectively, using 10 mmol of vinyl acetate as the acyl donor.

After hydrolysis of the acetate derivatives with potassium hydroxide in methanol, the enantiopure alcohols were identified as (*R*)-(+)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (*R*)-**3** ($[\alpha]_D^{20} = +21^\circ$ (*c* 2.7 in $CHCl_3$), >99% *ee*) and (*R*)-(+)-1-(4′-chlorophenyl)-2-phenylethanol (*R*)-**4** ($[\alpha]_D^{20} = +22^\circ$ (*c* 1.6 in $CHCl_3$), 97% *ee*), respectively. In general, the results obtained for alcohol (*R*)-**4** are moderate owing to its low solubility which decreases the transformation.

3.2.2.2. Lipase-mediated hydrolysis. Herein we report on the enzyme mediated enantioselective hydrolysis of acetates 1-(4′-chlorophenyl)-1-cyclopropyl methyl acetate (**5**) and 1-(4-chlorophenyl)-2-phenylethyl acetate (**6**) [14].

The racemic acetate derivatives were prepared by adding the corresponding alcohols to a solution of acetic anhydride in dichloromethane:methanol (1:1). The acetates were then transformed by lipases dissolved in a mixture of 1,4-dioxane and different concentrations of buffer solution (see Scheme 3). The results of the enzymatic hydrolysis are summarized in Table 3.

The 1-(4′-chlorophenyl)-1-cyclopropyl methyl acetate (**5**) was transformed to (*R*)-(+)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (*R*)-**3** with a high *ee* of 98% with a conversion of 47% using CRL as catalyst and 67% of buffer. For 1-(4′-chlorophenyl)-2-phenyethanol (**4**) the enantiomer obtained was (*R*)-(+)-1-(4′-chlorophenyl)-2-phenyethanol (*R*)-**4** in only one case, using CRL as biocatalyst with 20% aqueous buffer. The low solubility of this compound makes it very difficult for the reaction to take place with moderate amounts of water (necessary for the hydrolysis reaction). Thus, the final product (*R*)-**4** with high enantiomeric excess could not be obtained by means of this methodology.

4. Detoxification by *B. cinerea*

The biotransformation of the fungistatic agent 1-(4′-chlorophenyl)-2-phenyethanol (**4**) by the phytopathogen *B. cinerea* has been studied in a previous report [14]. In incorporation experiments of **4** at a concentration of 150 ppm, *B. cinerea* was unable to detoxify this compound. When biotransformation was carried out at a lower concentration, the ketone derivative was obtained as the only product, benzyl 4′-chlorophenyl ketone (**2**), which showed less antifungal activity than the corresponding alcohol **4** against the fungus *B. cinerea* [10], suggesting that the fungus has its own detoxification mechanism.

Thus, in order to investigate the detoxification mechanisms for antifungal compound **3**, the biotransformation of that alcohol by *B. cinerea* UCA992 was studied.

4.1. Detoxification of (±)-1-(4′-chlorophenyl)-1-cyclopropyl methanol (3) by B. cinerea UCA992

Biotransformation was carried out on surface cultures using Roux bottles for five days, after which the mycelium was filtered, extracted with ethyl acetate and the extract dried over anhydrous sodium sulphate. In addition to the starting material (see Scheme 4), the purification of the organic dry extract afforded two compounds: 4′-chlorophenyl cyclopropyl ketone (**1**), and compound **7**, a mixture of diastereoisomers 50:50 of the corresponding dimer of the biotransformation starting material which could be separated by HPLC giving *syn*-**7** (>99% *ee*) and *anti*-**7** (30% *ee*). The presence of these compounds as biotransformation products shows the interesting enzymatic potential of the fungus *B. cinerea* UCA992 as a biocatalyst.

As mentioned above, apart from the ketone **1**, the new compound **7** was isolated from the biotransformation. Many of the signals in their ^1H and ^{13}C NMR spectra were similar to those of 1-(4′-chlorophenyl)-1-cyclopropylmethanol (**3**). However, the signal corresponding to the hydroxyl group on C-1 was absent while the signal assigned to H-1 was more deshielded, indicating that the hydroxy group of **3** had been etherificated with another molecule of the alcohol. In addition, the HRMS of the compound confirmed the structure of the new product as di(4′-chlorophenyl cyclopropyl methyl)ether (**7**). To confirm that this compound was a biotransformation product and not a condensation product from the chemical reaction between two molecules of the starting material of the biotransformation, **3**, we treated 1-(4′-chlorophenyl)-1-cyclopropylmethanol (**3**) in the culture broth under the same conditions as those of the biotransformation, but without the fungus. No condensation compound was obtained.

The antifungal activity of the biotransformation products against the fungus *B. cinerea* was studied and proved to be less active than the alcohol used as substrate (for product **1** [10]) or completely inactive (compound **7**). This fact confirms the existence of a detoxification mechanism by the phytopathogen.

5. Conclusions

The studies described above have demonstrated the potential of several methods, biocatalytic and chemical, to obtain enantiomerically pure forms of 1-(4′-chlorophenyl)-1-cyclopropyl methanol (**3**) and 1-(4′-chlorophenyl)-2-phenylethanol (**4**), which had already proven to be highly active against *B. cinerea* [10].

The best selectivity-yield relationship for the reduction of benzyl 4′-chlorophenyl ketone (**2**) was observed after treatment with oxazaborolidine [17], yielding compounds (*R*)-**4** and (*S*)-**4** [14], with >99% *ee* and 98% yield. However, results for ketone **1** were only moderate using this methodology.

We were thus able to obtain enantiomerically pure alcohols of 1-(4′-chlorophenyl)-1-cyclopropyl methanol (**3**) by means of biocatalytic methods. The best result for the preparation of (*S*)-**3** (96% *ee*) was observed after fermentation of 4′-chlorophenyl cyclopropyl ketone (**1**) with baker's yeast cells in the presence of allyl alcohol and hexane, while the best *R* selectivity-yield relationship for compound **3** was after lipase-mediated hydrolysis of 1-(4′-chlorophenyl)-1-cyclopropyl methyl acetate (**5**) to give (*R*)-**3** with 98% *ee*.

Compounds (*R*)- and (*S*)-1-(4′-chlorophenyl)-2-phenylethanol (*R*)- and (*S*)-**4** were also prepared by enzymatic methods with excellent enantioselectivity results. Thus, (*S*)-**4** was obtained with 99% *ee* by means of baker's yeast reduction in the presence of addi-

tives while the best result for (*R*)-**4** (97% *ee*) was observed after lipase-mediated acetylation with PPL followed the hydrolysis of the acetate.

Based on previous results pointing to the high antifungal activity of enantiopure alcohols **3** and **4** [10] against the phytopathogen *B. cinerea*, and the detoxification mechanism exhibited by **4** [14], we also carried out the biotransformation of 1-(4′-chlorophenyl)-1-cyclopropyl methanol (**3**) by the fungus. Biotransformation products have been shown to be less toxic to fungal growth than **3**, confirming the existence of a detoxification mechanism by the phytopathogen. The main detoxification reaction involved oxidation of the starting material. It is also interesting to note the isolation of a new dimer compound of (±)-1-(4′-chlorophenyl)-1-cyclopropylmethanol (**3**) as a detoxification product by *B. cinerea* UCA992.

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